Diagnosis of Vaginitis: A review and update

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Objectives

- To understand the common causes of vaginitis
- To discuss laboratory methods to diagnose vaginitis
- To discuss coinfection of vaginitis and sexually transmitted infections

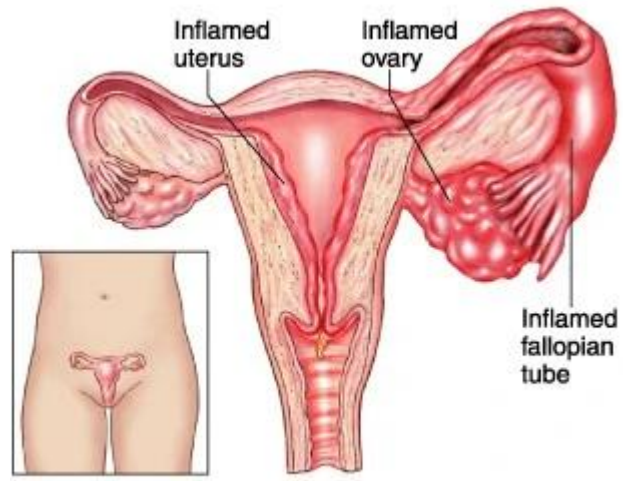
Vaginitis

- One of the most common gynecologic disorders
- Most common reason for women's visit to a health care provider
- 10 million office visits/ year and 7 % visits to gynecologists
- 1% antibiotics prescribed in ambulatory setting
- Approx 50% of women treated without a proper diagnosis
- Risk factors include low socioeconomic status, new or multiple sex partners, drug use, smoking, antibiotics use, vaginal douching etc.

Paladine HL, Desai UA. Vaginitis: Diagnosis and Treatment. Am Fam Physician. 2018 Mar 1;97(5):321-329

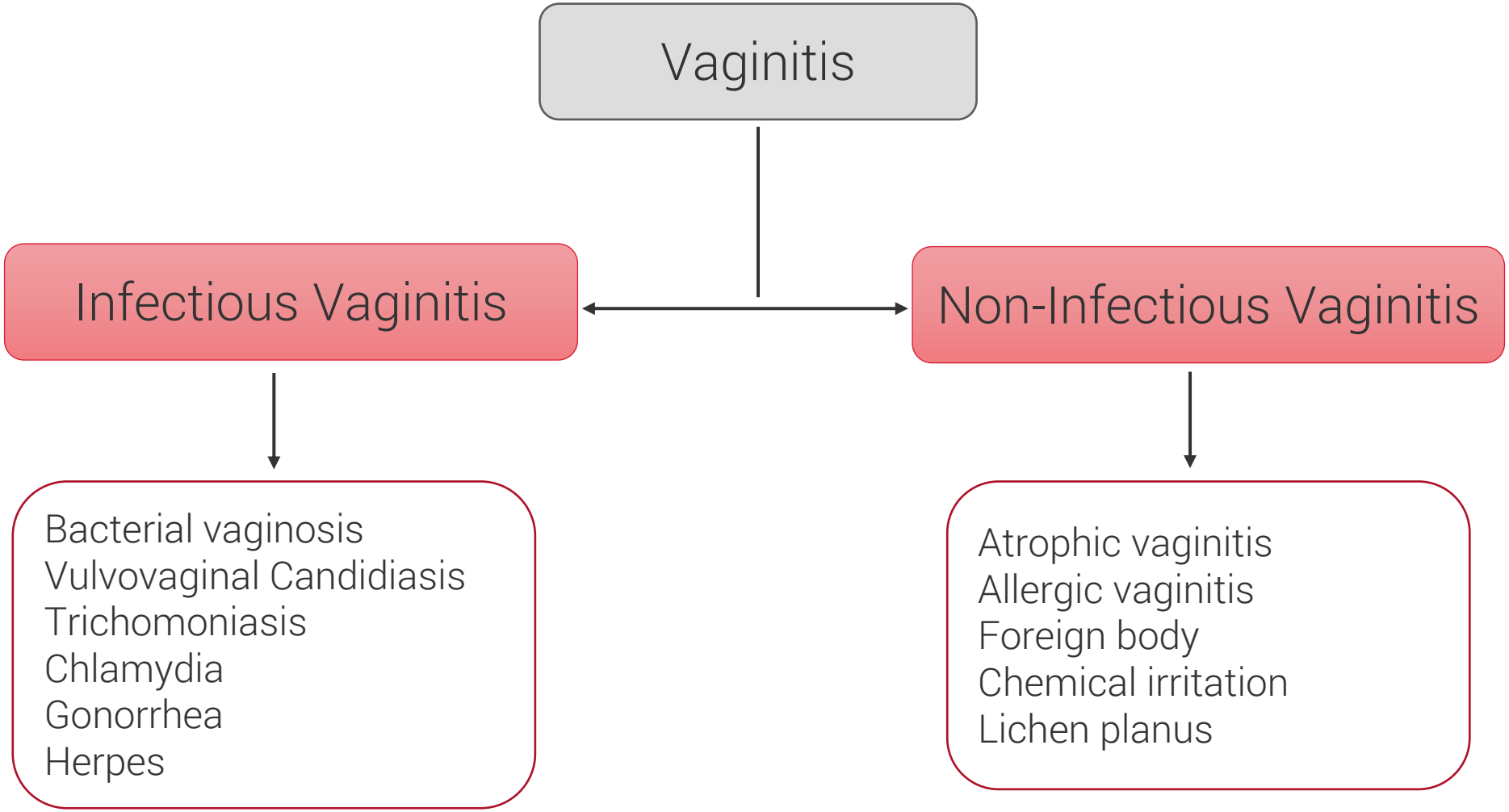
What is Vaginitis?

- Clinical syndrome caused by inflammation/infection of the vagina
- Characterized by abnormal vaginal discharge
  - » Clear, gray/frothy green/curdy or cheesy with or without odor
- Sometimes caused by a sexually transmitted infection
- Associated with acquisition of HIV and other STIs
- Linked to endometritis, pelvic inflammatory disease
- Adverse outcomes: infertility, preterm birth, low birth weight



https://americanpregnancy.org/womens-health/pelvic-inflammatory-disease

Causes of Vaginitis



Causes of Vaginitis

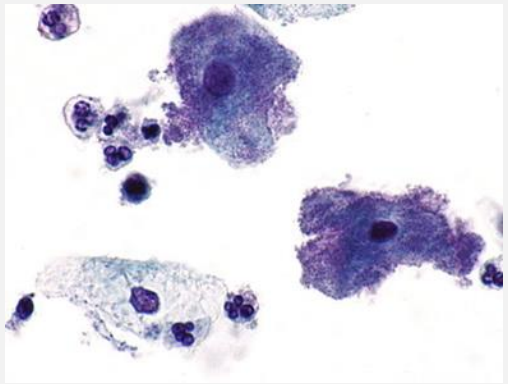
TABLE 1

Signs and Symptoms of Vaginitis

Diagnosis	Etiology	Symptoms	Signs	Other risks
Bacterial vaginosis	Anaerobic bacteria *(Prevotella, Mobiluncus, Gardnerella vaginalis, Ureaplasma, Mycoplasma)*	Fishy odor; thin, homogenous discharge that may worsen after intercourse; pelvic discomfort may be present	No inflammation	Increased risk of HIV, gonorrhea, chlamydia, and herpes infections
Vulvovaginal candidiasis	*Candida albicans,* can have other *Candida* species	White, thick, cheesy, or curdy discharge; vulvar itching or burning; no odor	Vulvar erythema and edema	—
Trichomoniasis	*Trichomonas vaginalis*	Green or yellow, frothy discharge; foul odor; vaginal pain or soreness	Inflammation; strawberry cervix	Increased risk of HIV infection Increased risk of preterm labor Should be screened for other sexually transmitted infections
Atrophic vaginitis	Estrogen deficiency	Thin, clear discharge; vaginal dryness; dyspareunia; itching	Inflammation; thin, friable vaginal mucosa	—
Irritant/allergic vaginitis	Contact irritation or allergic reaction	Burning, soreness	Vulvar erythema	—
Inflammatory vaginitis	Possibly autoimmune	Purulent vaginal discharge, burning, dyspareunia	Vaginal atrophy and inflammation	Associated with low estrogen levels

Am Fam Physician. 2018;97(5):321-329

Infectious Vaginitis Etiologies

Bacterial Vaginosis (BV)
30 -50% of cases *Gardnerella vaginalis, Atopobium vaginae, Megasphaera, Prevotella spp,* and other anaerobes   

Co-infections common: 20 -30%

Vaginitis Differentiation

	Normal	Bacterial Vaginosis	Candidiasis	Trichomoniasis
Symptom presentation		Odor, discharge, itch	Thick discharge, itch, discomfort, dysuria	Discharge, itch, 50% asymptomatic
Vaginal discharge	Clear to white	Homogenous, adherent, thin, milky white; malodorous "foul fishy"	Thick, clumpy, white "cottage cheese"	Frothy, gray or yellow-green; malodorous
Clinical findings		No obvious inflammation	Edema and erythema	Cervical petechiae "strawberry cervix"
Vaginal pH	3.8-4.2	> 4.5	Usually, ≤ 4.5	> 4.5
KOH "whiff" test	Negative	Positive	Negative	Often positive
Saline wet mount	Lactobacilli	Clue cells (≥20%), no/few WBCs	Few WBCs	Motile flagellated protozoa, many WBCs
KOH wet mount			Pseudohyphae or spores if non-albicans species	

Treatment for Vaginitis

Bacterial Vaginosis

- Metronidazole (Flagyl), 500 mg orally twice daily for seven days OR
- Metronidazole 0.75% gel (Metrogel), one full applicator (5 g) intravaginally daily for five days
- Alternative with Tinidazole or Clindamycin

Candida vaginitis

- Topical azole regimens, over the counter or prescription
- Oral fluconazole

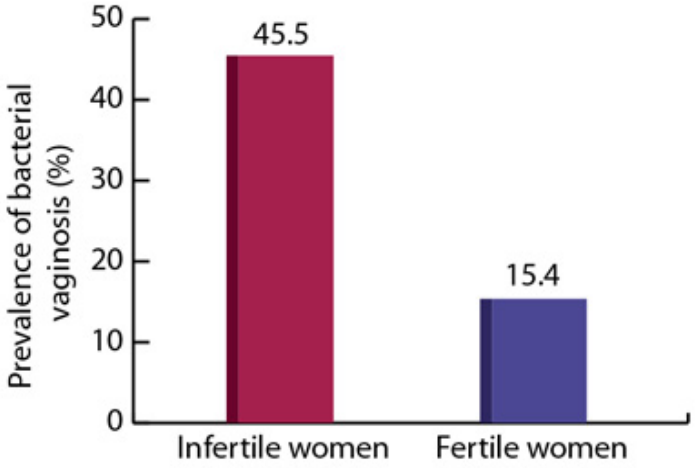
Trichomoniasis

- Metronidazole 500 mg orally twice daily for 7 days OR
- Tinidazole 2 g orally in a single dose

Major players of vaginitis

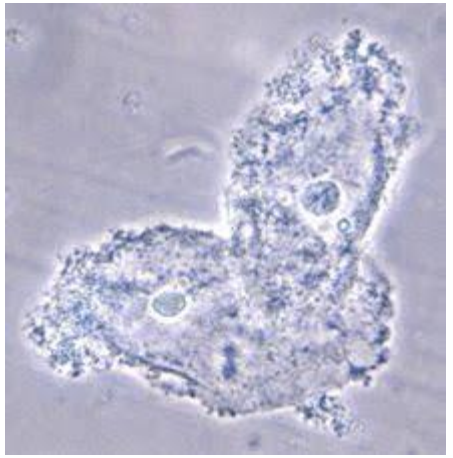
Bacterial Vaginosis (BV)

- Most common vaginal condition in women ages 15-44
- Prevalence in the United States is estimated to be 21.2 million (29.2%)
- Most women found to have BV (84%) are asymptomatic
- Higher rates in women of color (African-American 51%, Hispanic 32%) than whites (23%)
- Associated with infertility



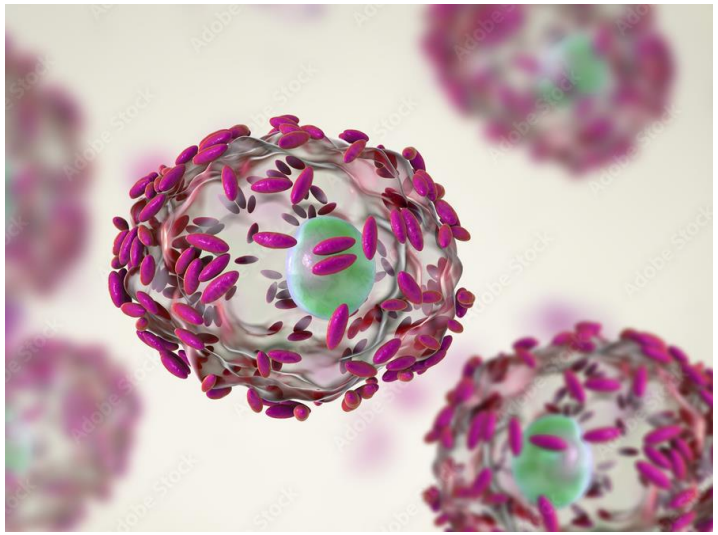
https://www.ashasexualhealth.org/

BV: Clinical Presentation



- Vaginal discharge, thin, grey, homogenous
- Malodorous (fishy) smell
- pH >4.5, positive for amine (Whiff test), presence of clue cells
- Asymptomatic or mild irritation

Risk factors for BV



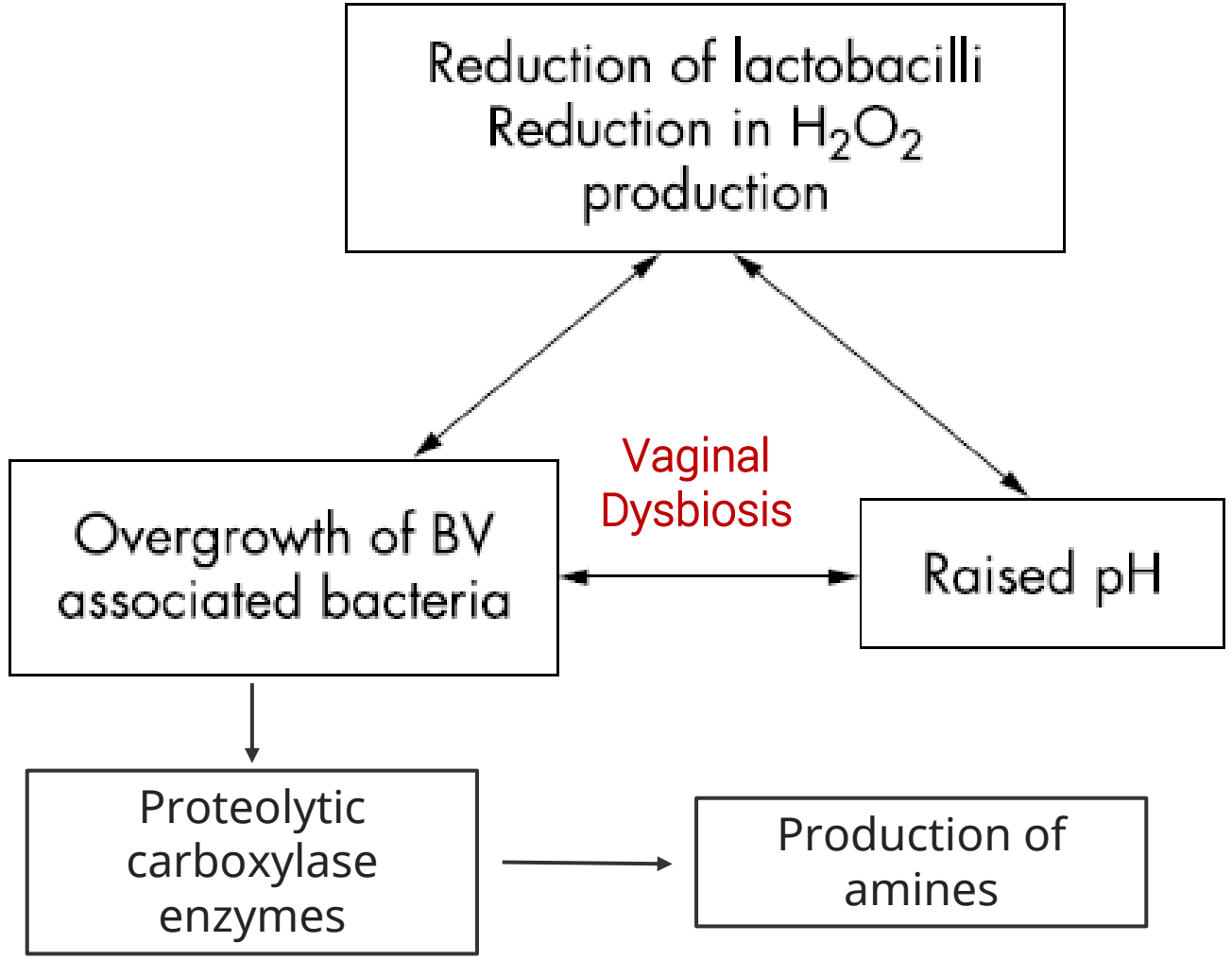
- Sexual intercourse, multiple or new partners
- Vaginal douching
- Intrauterine device use
- Recent antibiotic use
- Smoking
- Women testing positive with BV were coinfected with an STI (chlamydia, gonorrhea, trichomonas)
- Women with vaginitis symptoms are at risk for an STI, HIV, herpes
- CDC recommends testing women with BV for STIs

Factors involved in BV pathogenesis

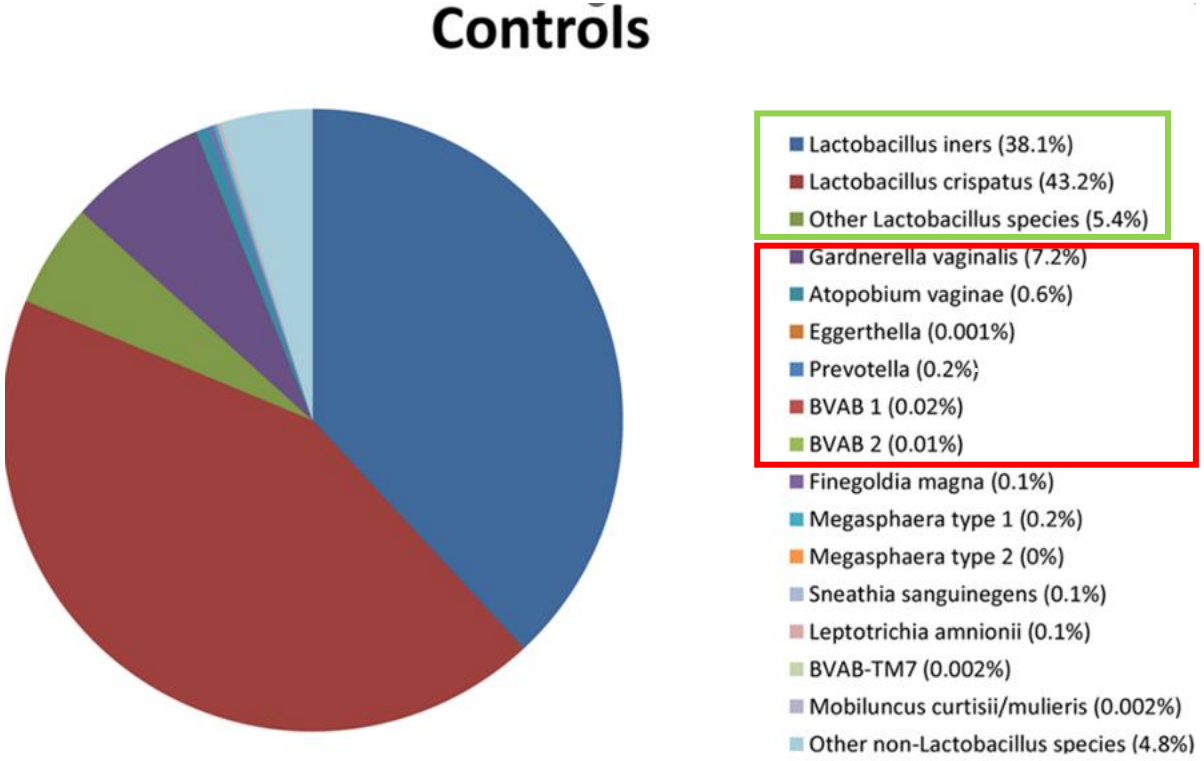
Good bacteria
Lactobacilli sp
(L. crispatus and L. jensenii)

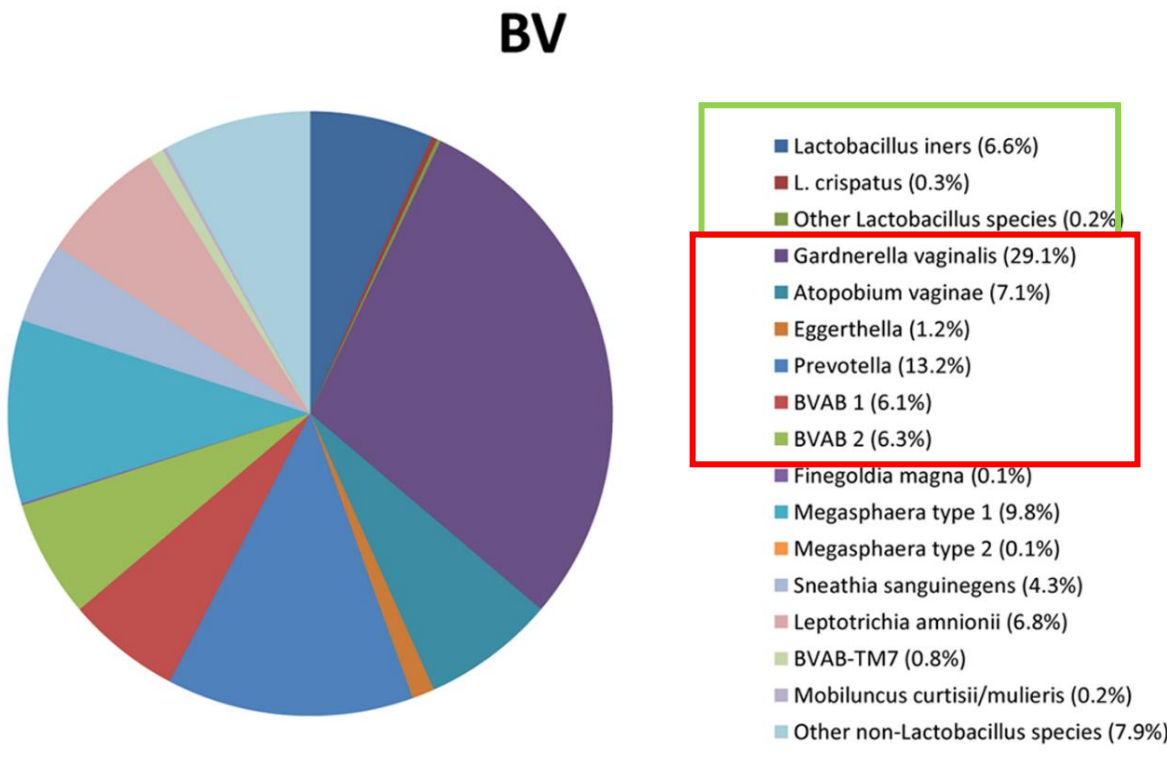
Lactic acid and H_2O_2

Bad bacteria
Gardnerella vaginalis
Atopobium vaginae (renamed *Fannyhessea vaginae*)
Prevotella sp
Mobiluncus
Megaspheaera



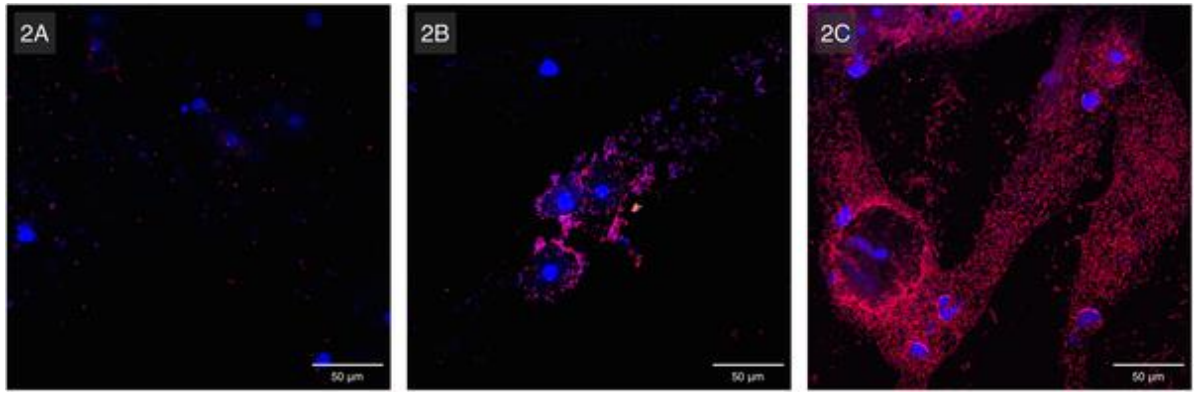
BV: multiple organism syndrome

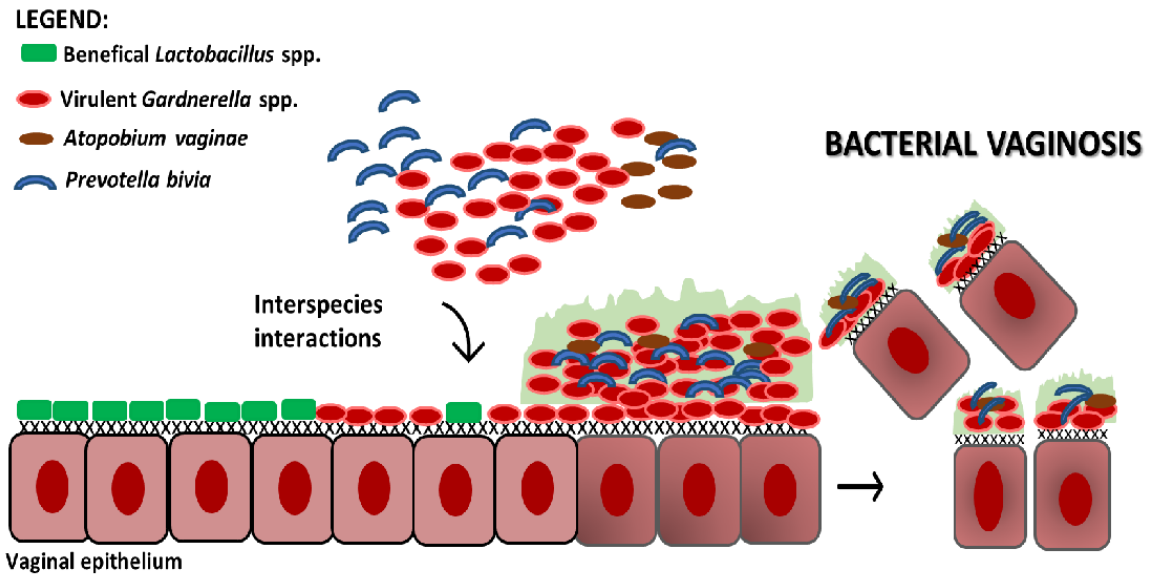




Factors involved in BV pathogenesis

- *G. vaginalis* is a key player in the pathogenesis of BV
- Adheres to vaginal epithelium first and provide scaffold for other species
- Biofilm development due to sialidase enzyme activity





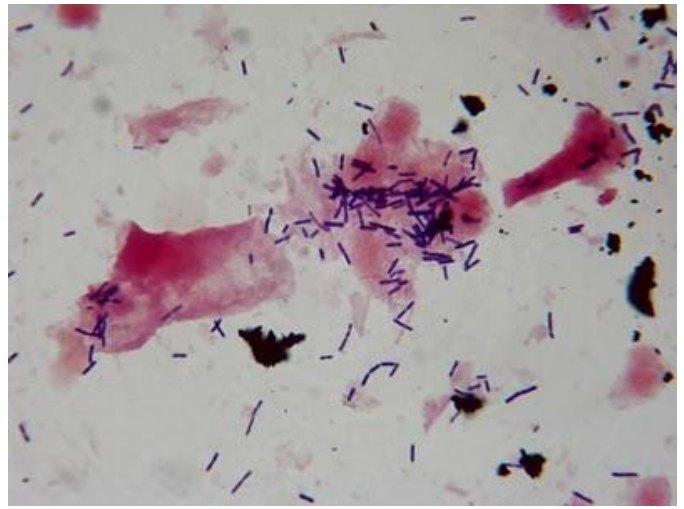
Verstraelen H, et al. Curr Opin Infect Dis. 2013 Feb;26(1):86-9
Castro et al Pathogens 2021, 10(2)

Complications of BV

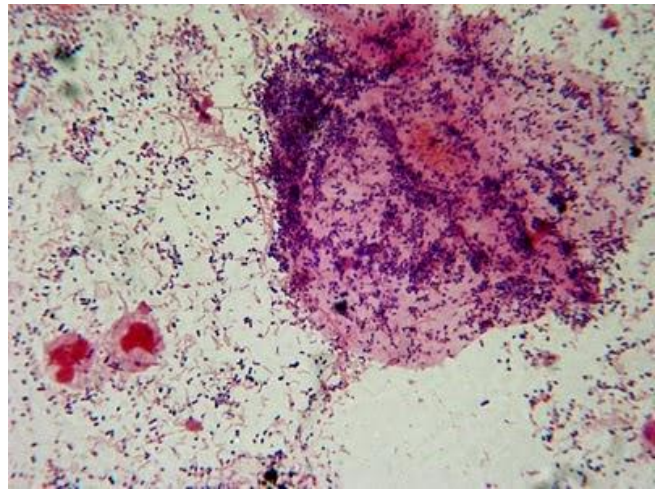
Preterm delivery, premature rupture of membranes
Increased risk of Pelvic inflammatory disease
Increased susceptibility to HIV acquisition/transmission, other STIs
Persistence of HPV infection
Recurring rates 25% within 4-6 weeks after treatment

BV Diagnostic approaches

- Vaginal gram stain (Nugent score)
- Point of care Assays
  - » Amsel Diagnostic Criteria
  - » OSOM BV Blue
- Nucleic acid amplification tests



Normal vaginal flora. Gram stain showing epithelial cells and Gram-positive lactobacilli (purple rods)



BV. Gram stain showing clue cells with Gram variable coccobacilli

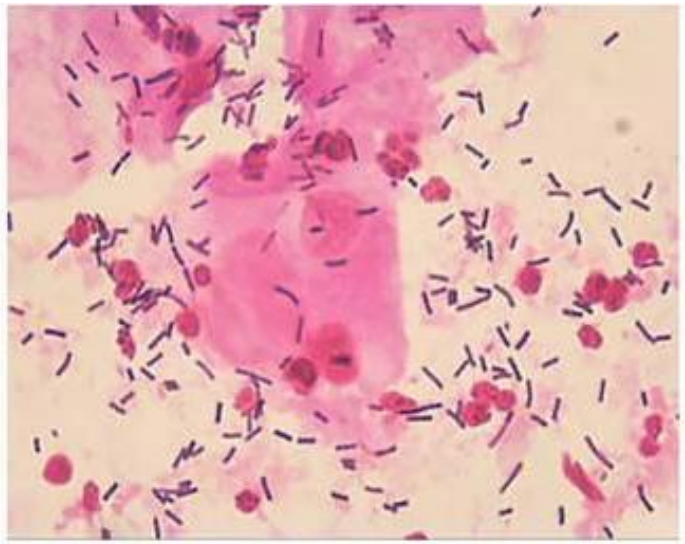
Vaginal Gram's Stain with Nugent Scoring

- Gold Standard method
- Gram stain scoring system for vaginal swabs
- Assess presence of large Gram-positive rods (*Lactobacillus* sp), small Gram variable rods (*Gardnerella vaginalis*), and Gram variable curved bacilli (*Mobiluncus*)
- Sensitivity of 89.1% , specificity of 83.1%.
- Time consuming, trained microscopists

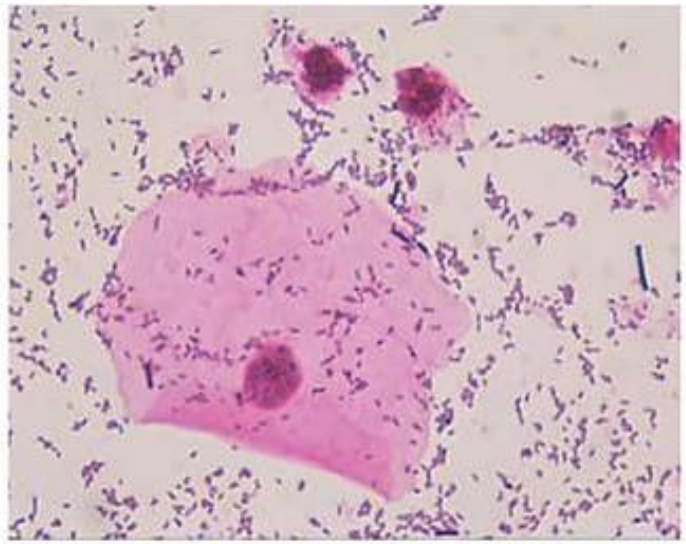
Vaginal Gram Stain with Nugent Scoring

Nugent Scoring System (0-10) for Gram-Stained Vaginal Smears					
Score	*Lactobacillus* morphotypes		*Gardnerella* and *Bacteroides* morphotypes		Curved gram-variable rods
0	4+		0		0
1	3+		1+		1+ or 2+
2	2+	+	2+	+	3+ or 4+
3	1+		3+		-
4	0		4+		-

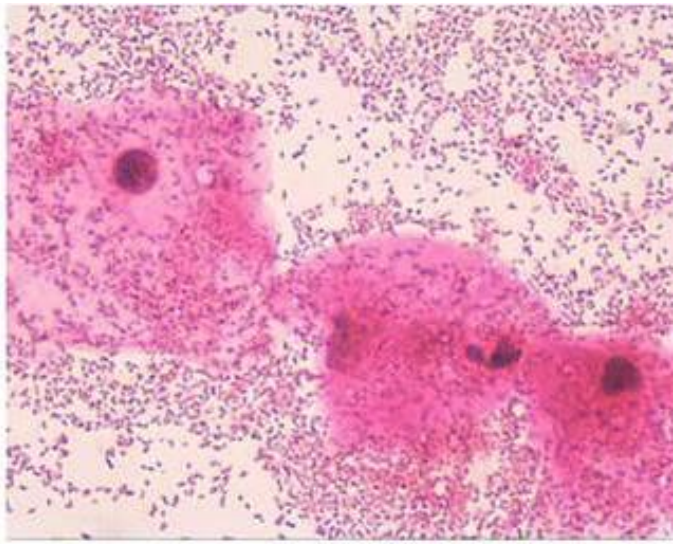
Scoring Based on Morphotypes per High Power Field: 0 = 0; 1+ = <1; 2+ = 1-4; 3+ = 5-30; 4+ = >30
Total Score: 0-3 Normal; 4-6 Intermediate; 7-10 Bacterial Vaginosis



(i) Normal Vaginal Flora (0-3)



(ii) Altered Vaginal Flora (4-6)



(iii) BV (7-10)

https://www.std.uw.edu/go/comprehensive-study/vaginitis/core-concept

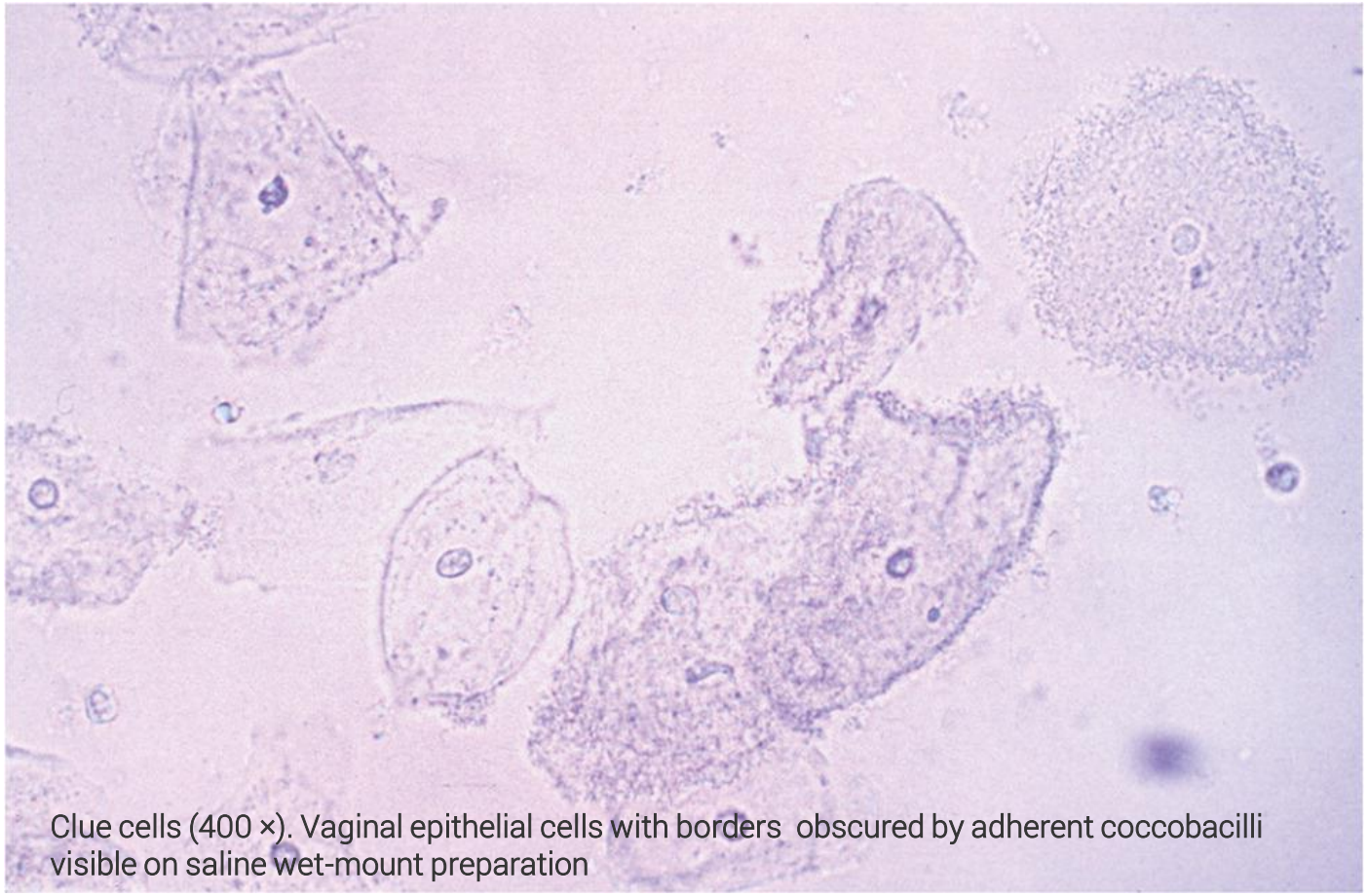
Amsel Diagnostic Criteria for BV

- Three out of four criteria must be met

- Thin, homogenous discharge
- Positive whiff test (amine odor produced by added 10% KOH)
- Clue cells present on microscopy
- Vaginal pH >4.5

- Sensitivity 37%-70% and Specificity 94% to 99%, respectively, compared to the gold-standard Gram stain Nugent score, moderate reproducibility

Vaginal epithelial cells with adherent coccobacilli



Clue cells (400 ×). Vaginal epithelial cells with borders obscured by adherent coccobacilli visible on saline wet-mount preparation

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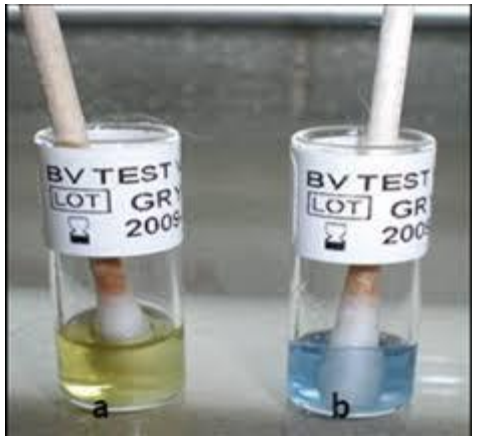
Other point-of-care BV assays

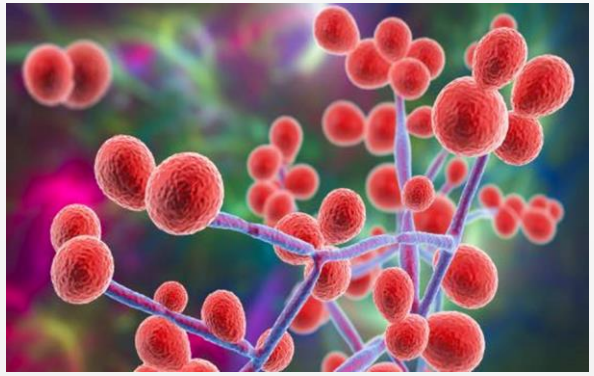


- OSOM BVBlue Test
  - » CLIA-waived
  - » Detects elevated vaginal fluid sialidase
  - » Results in 10 Minutes
  - » Sensitivity 92.8%, Specificity 98% compared to Nugent score
  - » Does not rule out other vaginitis or STI causes

Candida vaginitis

Vulvovaginal candidiasis (VVC) or Candida vaginitis

- Caused by various *Candida* spp.
  - » *C. albicans* 75-90%, *C. glabrata* 5-10%, *C. tropicalis* 5-10%



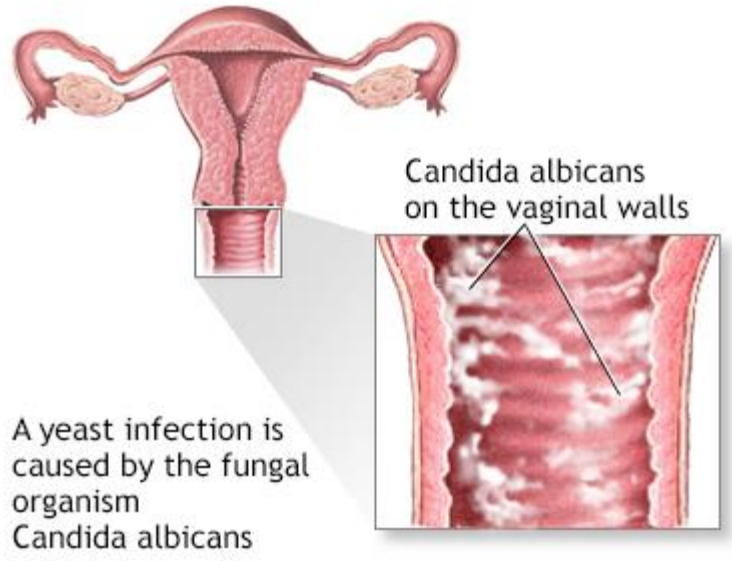
- 10-20% of women can be colonized
- In U.S. 13 million cases per year, often underreported
- Affects 70-75% of women during their lifetime, with 40-50% having at least 1 recurrence
- Risk factors include hormonal changes, pregnancy, uncontrolled diabetes, recent antibiotic use, pregnancy, HIV infection, steroids

VVC-Risk factors and etiology

- **Not sexually transmitted**
- Many women are colonized by yeast as part of normal flora
- *C. albicans* 90% of cases, *C. glabrata* and other non-albican *Candida* spp. 5-15%
- Often over-diagnosed in prepubescent and post-menopausal individuals
- Unknown why some women develop symptoms and others remain asymptomatic
- Not associated with reduction in lactobacilli in vaginal microbiome

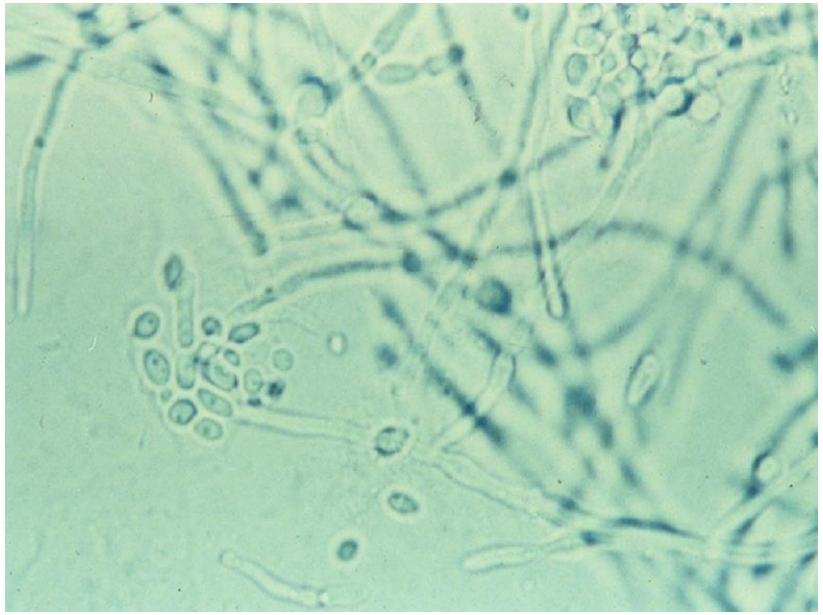
Clinical features of VVC

- Abnormal vaginal discharge, cheesy or curd-like, clumpy, white, and odorless
- Vaginal soreness, edema, fissures, and excoriations may be seen
- Vulvar burning or itching, redness
- Dysuria, dyspareunia
- Flare prior onset of menses

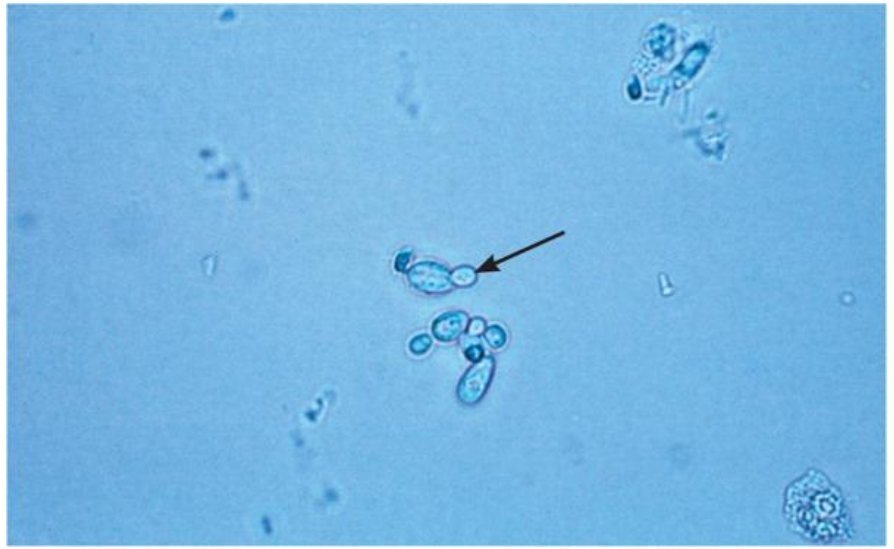


VVC- Traditional Diagnostics

- Clinical signs and symptoms plus positive saline/KOH microscopy
- Budding yeast or hyphae
- Normal acidic vaginal pH (<4.5)
- Wet mount sensitivity 43.9% - 78%, specificity 75% - 88.9%



Wet prep using KOH shows branching pseudohyphae and yeast buds



Budding yeast

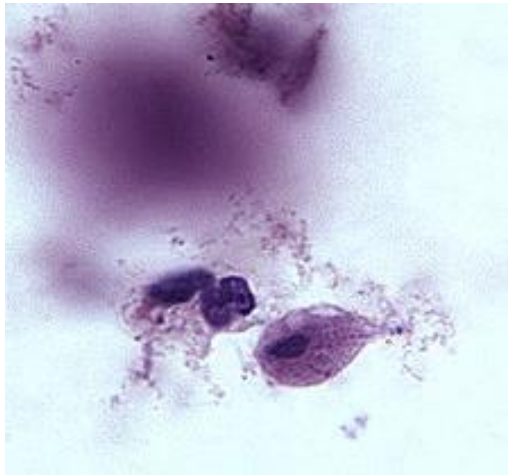
VVC- Traditional Diagnostics

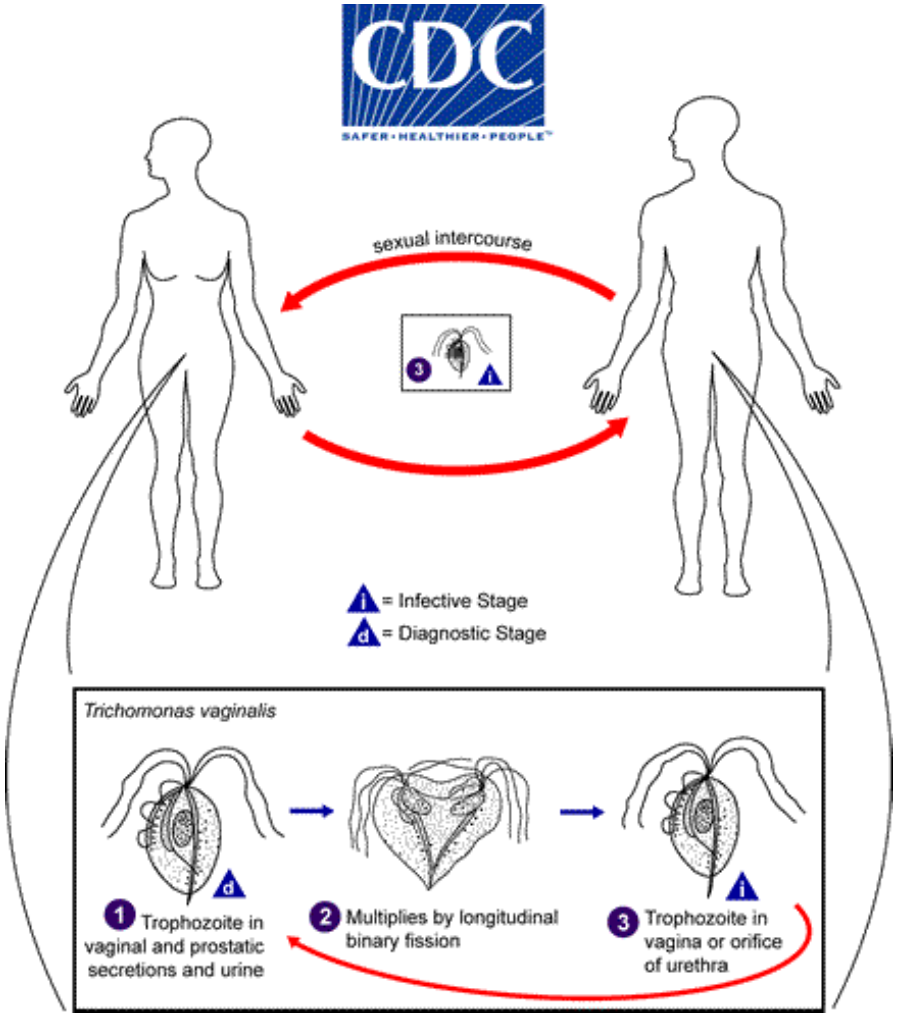
- Fungal culture: Gold standard
  - » If vaginitis symptoms, but no fungal elements seen on microscopy
  - » If vaginitis is persistent or recurrent
  - » Identify azole-resistant strains *C. glabrata, C. krusei* (intrinsically resistant to fluconazole)*
  - » *C. glabrata* is missed on microscopy

Trichomonas vaginalis

Trichomonas vaginalis

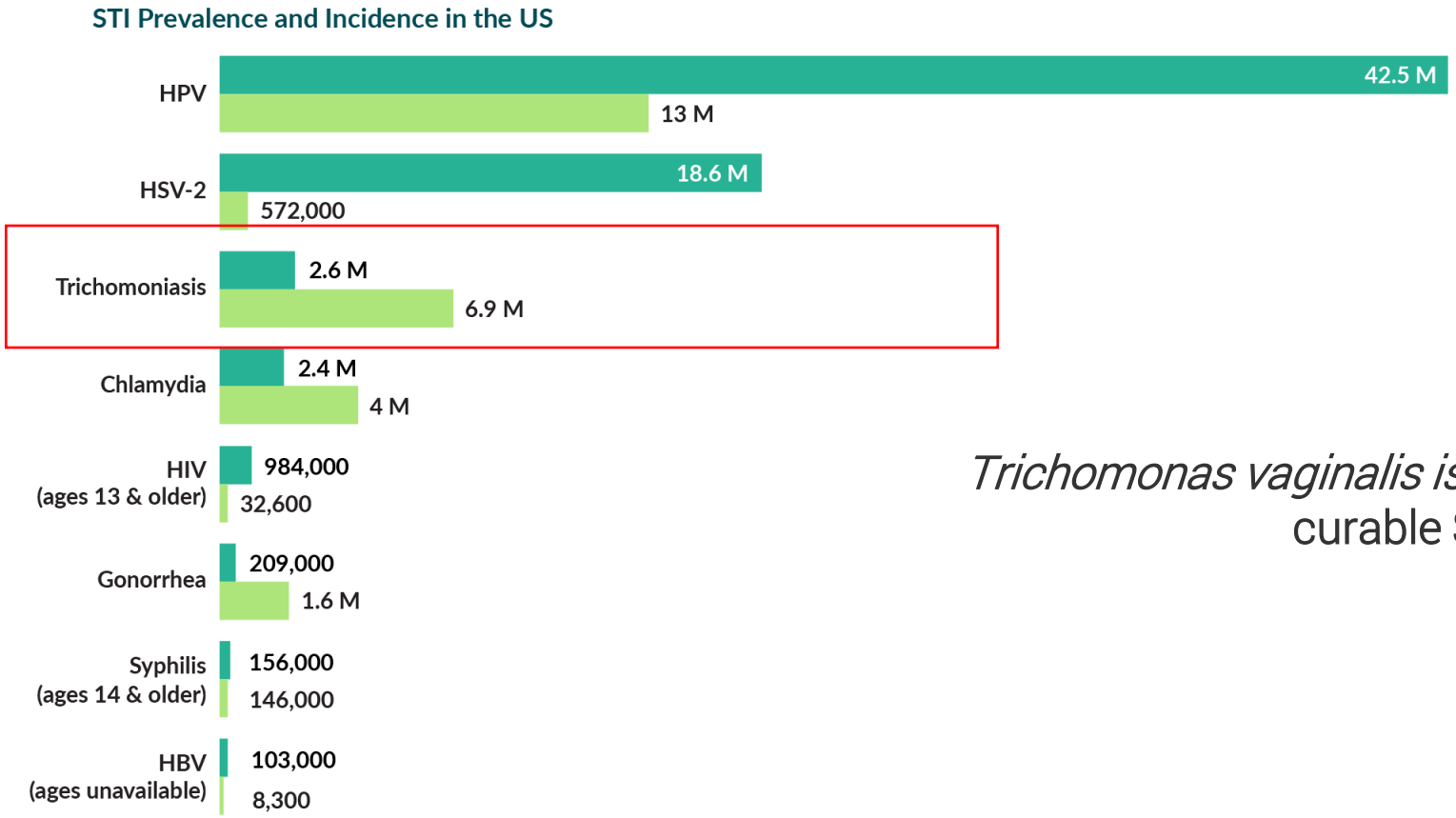
- Most common curable STI
- Human are the only known hosts
- Vaginitis, cervicitis, PID
- Motile, flagellated protozoan





https://www.cdc.gov/dpdx/trichomoniasis/index.html

Estimated prevalence of STIs in the US

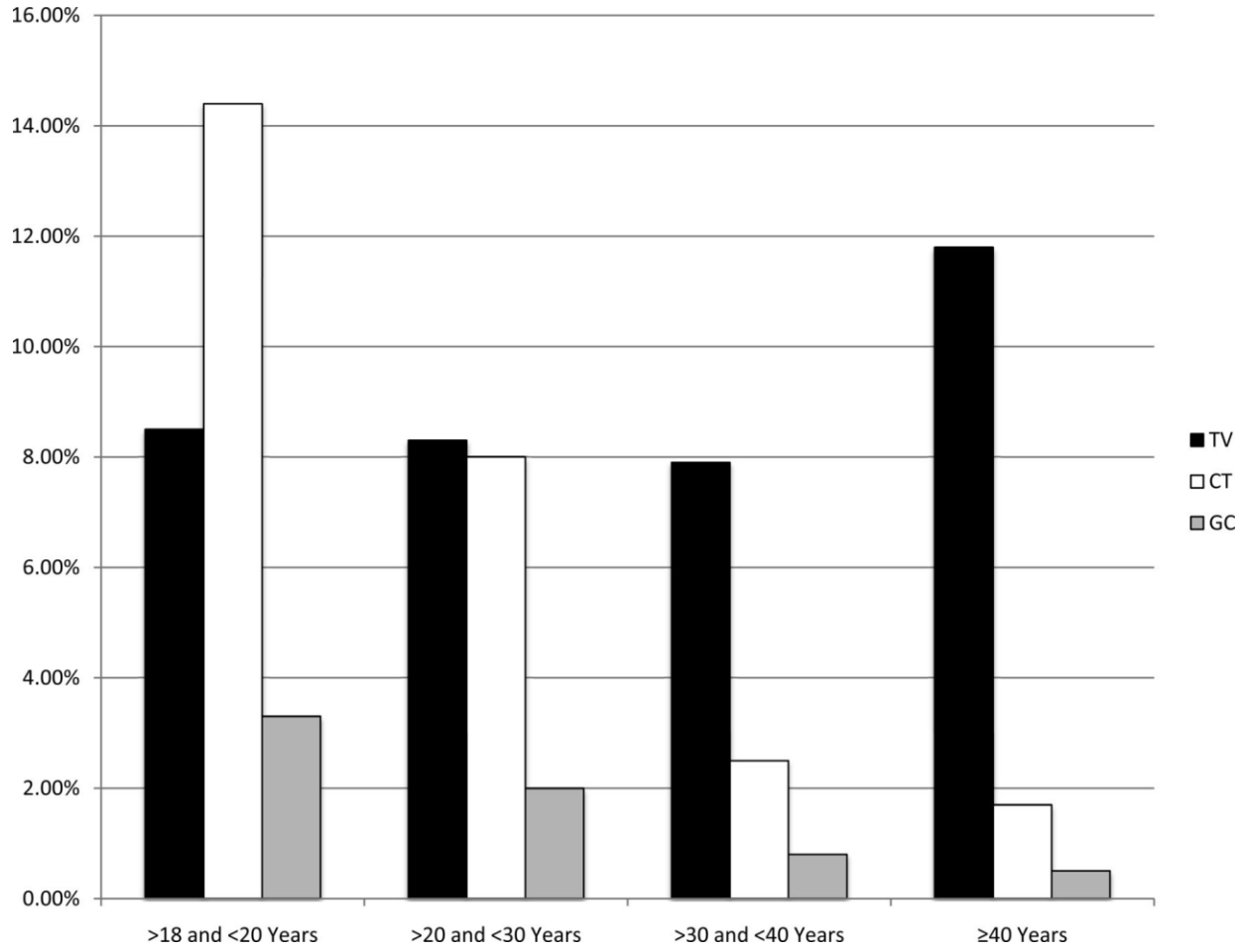


*Bars are for illustration only; not to scale, due to wide range in number of infections. Estimates for adults and adolescents ages 15+ unless otherwise stated. HIV and HBV data only represent sexually acquired infections.

Trichomonas vaginalis is the most common curable STI

csddc.org/resource/sti-cost-estimates-us/

Trichomonas vaginalis - Prevalence



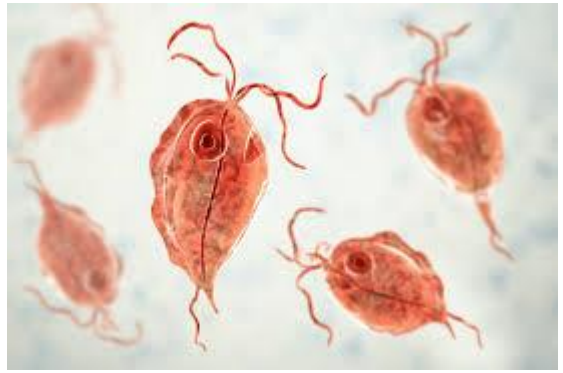
J Clin Microbiol. 2012Aug;50(8):2601-8

T. vaginalis: Clinical Presentation

- Majority are asymptomatic 50%-70%
- Symptoms
  - » Malodorous, purulent discharge
  - » Itching, burning sensation, bleeding, dyspareunia, dysuria
  - » Lower abdominal pain
  - » Vulvovaginal erythema
  - » Strawberry cervix

https://www.cdc.gov/std/treatment-guidelines/trichomoniasis

Trichomonas vaginalis



- Indications for testing
  - » Women seeking care for vaginal discharge
  - » High risk of infection (new/multiple partners, history of STDs, commercial sex)
  - » Serious adverse outcomes occur primarily among pregnant women and individuals at high risk for sexually transmitted diseases

https://www.cdc.gov/std/treatment-guidelines/trichomoniasis.

Consequences of untreated trichomoniasis

- Concurrent STIs, including chlamydia, gonorrhea and HSV types 1&2
- Increased time to clear HPV infections
- Possible connection with preterm birth and low birth weight
- Increased risk of HIV transmission
- Pelvic Inflammatory Disease (PID), Endometritis

TV-Traditional Diagnostics

- Saline wet mount (vaginal, urethral discharge, urine, prostatic secretions)
- pH >4.5, amine test positive
- No cyst stage, trophozoite: 8-15 µm, single nucleus, 4 anterior flagella
- Miss more than 50% of trichomoniasis
- Sensitivity 44%-68%, specificity 100%

Trichomonas vaginalis (400 ×)

https://www.cdc.gov/std/treatment-guidelines/trichomoniasis.

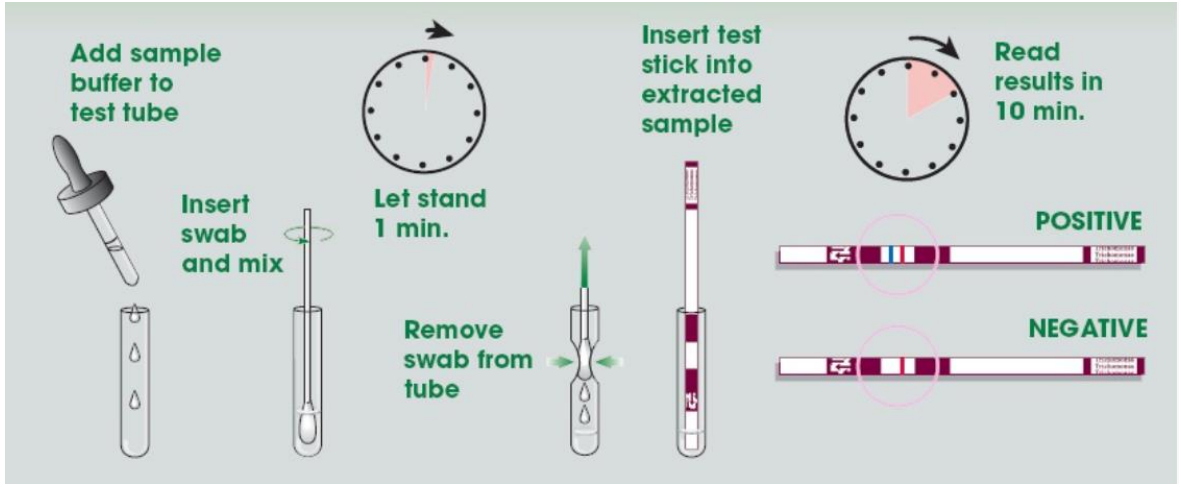
TV-Traditional Diagnostics

- Culture more sensitive than wet mount, not widely available
- Considered the gold standard method for diagnosing *T. vaginalis* infection before NAAT
- Vaginal secretions preferred
- Specimen inoculated within 1 hr of collection
- Requires incubation at 37°C
- Sensitivity 44%-75%, specificity of 100%

InPouch® kit or Diamond's medium, TAT 5-7 days, requires daily sampling for microscopic examination

TV-rapid diagnostics

- CLIA-waived, POC on vaginal specimens,
- Immunochromatographic assay to detect Trich antigens
- Rapid 10-15 mins
- Sensitivity 82%-85%, Specificity 97%-100, compared to wet mount, culture, and NAAT



https://www.ridacom.com/en/products

TV- Molecular diagnostics

- The CDC recommends nucleic acid amplification testing (NAAT) for the detection of *T. vaginalis*
- Multiple FDA-cleared assays
  - » Hologic Aptima T. vaginalis NAAT
  - » Cepheid gene Xpert T vaginalis NAAT
  - » Roche Cobas TV/MG assay
  - » Abbott Alinity m STI assay (TV, GC, CT, MG)
- High sensitivity and specificity >95%-100%
- Cleared for use in women (vaginal, urine, endocervical), some approved for men (urine)

Molecular Diagnosis of Vaginitis

Molecular Diagnosis of Vaginitis

- Important for accurate diagnosis
- Common symptomatology for BV, VVC, and TV
- 40% of women with vaginitis leave an initial medical visit undiagnosed
- Traditional gram stain, culture, microscopy, subject to sampling, transport conditions, and technical competency and proficiency
  - » Reduced sensitivity compared to molecular diagnosis

Molecular Diagnosis of Vaginitis

- Nucleic acid testing
  - » PCR
  - » Transcription-mediated amplification
  - » DNA hybridization probe
- Testing of multiple targets
- Detect specific targets
- Single swab collection for other STIs

Direct Probe assays

- DNA probe binding to pathogen specific sequence
- Affirm VP III assay
  - » Moderately complex
  - » Detects *G. vaginalis*, *Candida* sp, and *T. vaginalis*
  - » Specimen collection device
  - » Results within 1 hr
- For BV, Sensitivity is 94% and specificity is 81% compared to Nugent score
- But *G. vaginalis* is the only target for BV
- For TV, sensitivity is around 70% compared to NAAT

NAAT for Vaginitis

- **FDA-cleared assays**
  - » PCR: BD MAX Vaginal Panel
  - » PCR: Xpert Xpress Vaginal Panel
  - » Transcription mediated amplification: Hologic Aptima BV, Hologic Aptima CV/TV
- **Laboratory developed tests** (NuSwab VG, OneSwab BV PCR Panel, SureSwab BV
- Symptomatic women only

NAAT for Vaginitis

- Differentially detect bacterial vaginosis through algorithmic analysis of lactobacilli and bacteria involved in BV (*G. vaginalis, A. vaginae, Megasphaera*-1 etc)
- Detect and differentiate between *Candida* spp with higher azole resistance
- Improved performance and diagnostic accuracy for the diagnosis of vaginitis
- High sensitivity, specificity, and negative and positive predictive values (98.7%, 95.9%, 92.9%, and 96.9%, respectively)
- Cost of molecular panels

Comparison of molecular methods

- PCR compared to probe-based assay

	Prevalence	TP	FP	TN	FN	Sensitivity		Specificity		PPV	NPV
						%	95% CI	%	95% CI	%	%
MAX VP-BV	41.6%†	76	4	99	3	96.2	89.3–99.2	96.1	89.8–98.7	95.0	97.1
Affirm-GV		76	19	84	3	96.2	89.5–99.2	81.6	72.7–88.5	80.0	96.6
MAX VP-*Candida*	32.1%	61	6	125	1	98.4	91.3–99.6	95.4	90.3–98.3	91.4	99.2
Affirm-*Candida*		43	0	131	19	69.4	56.4–80.4	100.0	97.2–100	100.0	87.3

Thompson A,. Eur J Clin Microbiol Infect Dis. 2020 Jan;39(1):39-44

Performance of NAAT testing for *T. vaginalis*

- Comparison of molecular methods for detection of *T. vaginalis* in symptomatic females
- 787 specimen pairs were analyzed by the Affirm VPIII and Aptima TV (ATV) assays

Diagnostic method	No. of true-positive results	No. of false-positive results	No. of false-negative results	No. of true-negative results	Sensitivity		Specificity		Predictive value (%)	
					%	95% CI	%	95% CI	Positive	Negative
Affirm VPIII	26	1	15	739	63.4	55.0–65.4	99.9	99.4–100	96.3	98.0
ATV	41	0	0	740	100	95.4–100	100	99.7–100	100	100

J Clin Microbiol. 2011;49(3):866-869.5

Vaginitis and other STIs

Coinfection of vaginitis and STIs

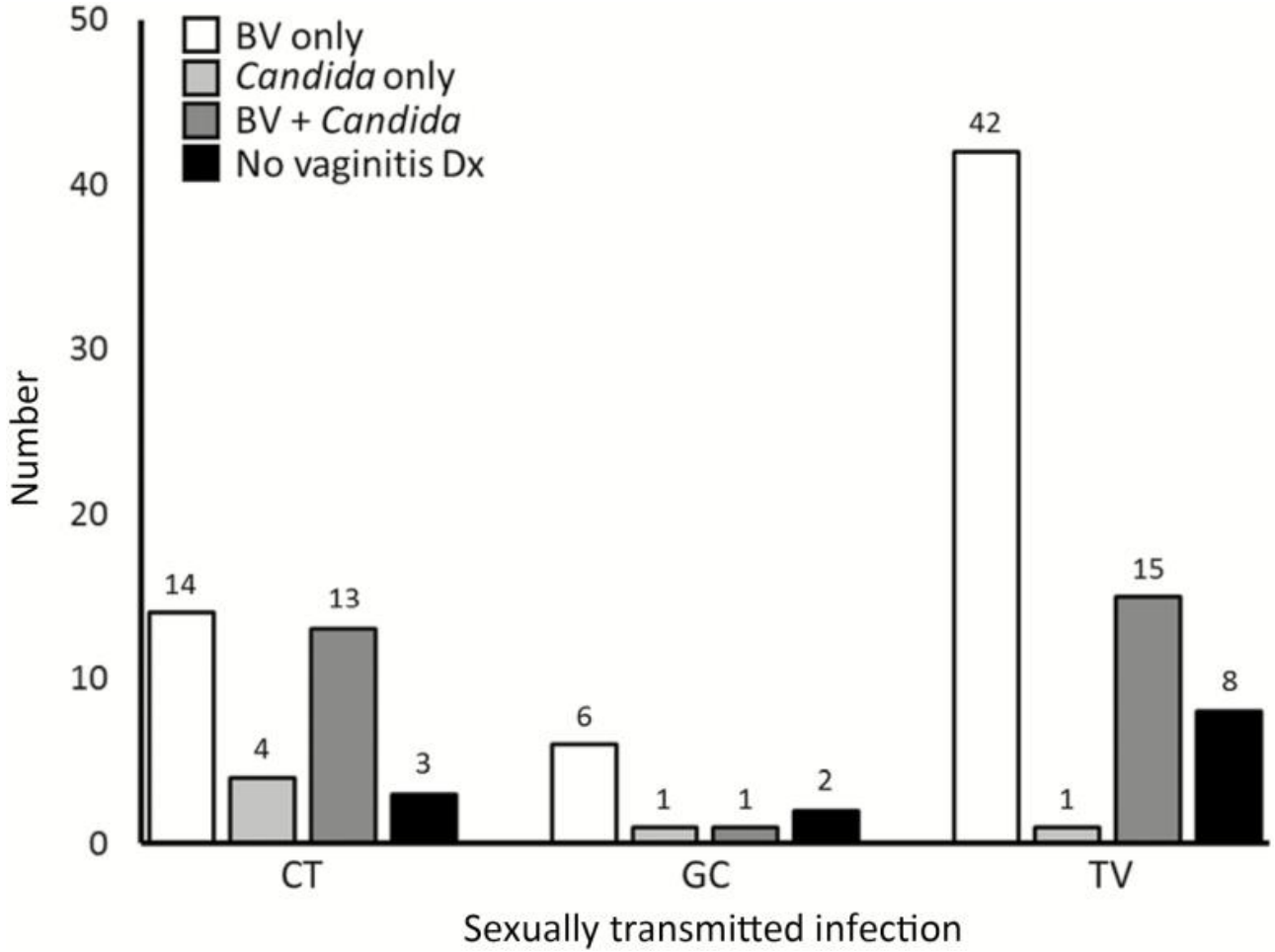
- Co-infection of vaginitis and STIs is common
- Approximately 20 %-30 % of women with bacterial vaginosis (BV) are coinfected with Candida species
- TV often co-exist with BV with a rate of 60-80%
- Approximately 25% of women with BV or VVC also had an STI
- Significant clinical, testing, and therapeutic implications

Sobel JD et al. Curr Infect Dis Rep. 2013;15
Van Der Pol B et al. Clin Infect Dis. 2019;68(3).

Coinfection of vaginitis and STIs

- 2019 study of STI detection rates in samples previously collected for vaginitis
- >85% of individuals positive for any STI were also positive for BV or *Candida* spp
- Women who were positive for BV were significantly more likely to have a CT or TV infection

Van Der Pol B et al. Clin Infect Dis. 2019;68(3)



- Women with symptoms of vaginitis could be at an increased risk for an STI
- Data supports comprehensive STI testing for women with vaginitis

Van Der Pol B et al. Clin Infect Dis. 2019;68(3)

Vaginitis clinical study

- 21 U.S. sites, 1051 women
- Nugent Score/Amsel Criteria, *Candida* spp. culture, TV culture and NAAT
- Vaginal swab for molecular testing for *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Mycoplasma genitalium*

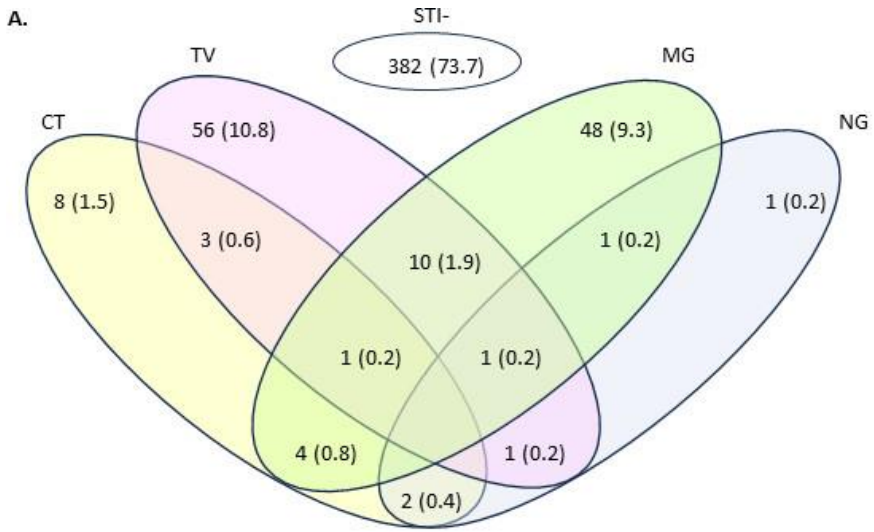
- 2/3 positive for BV/*Candida* spp/TV
  - » 36% positive for BV only, 16% yeast only, 2% TV only
  - » 18.5% had a mixed infections

- 45% of STIs are in women with BV (clinic diagnosed or lab diagnosed)
  - » Majority of STIs were MG and TV

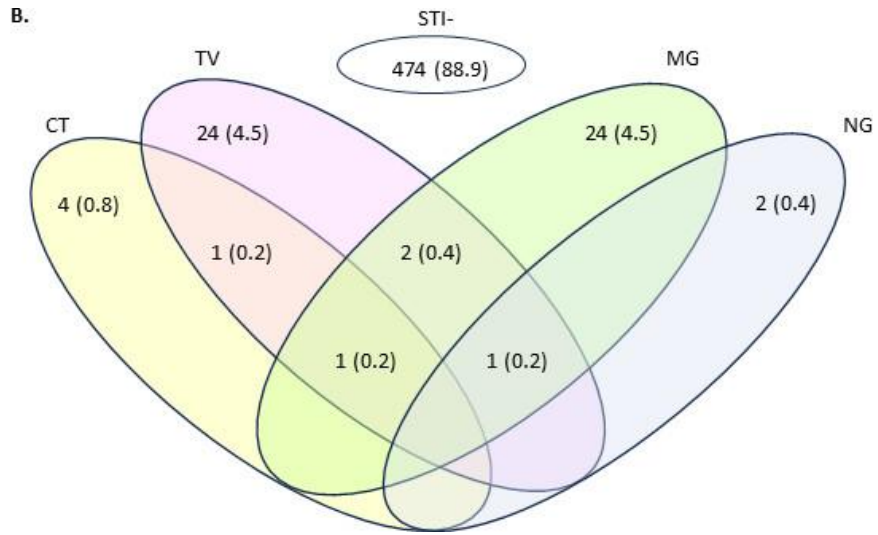
Schwebke JR J Clin Microbiol. 2024 Sep 11;62(9)

Vaginitis Clinical Study

BV positive



BV negative



STI infection in BV-positive women was 2-fold higher than in BV negative women
CT, TV, and MG were associated with BV (2-4-fold increase)

CDC recommends that all women diagnosed with BV be tested for STIs
Reflex test for other STI NAAT CT, NG, TV, and MG

Schwebke JR J Clin Microbiol. 2024 Sep 11;62(9)

Vaginitis: Take-home points

- Vaginitis is common with multiple etiologies
- BV and Trichomoniasis are associated with increased risk of HIV acquisition and transmission and other STI's
- Diagnosis is made using a combination of clinical findings, and office-based or laboratory testing
- Traditional diagnostic methods involve microscopy and culture
- NAAT assays are sensitive and specific for vaginitis
- Highly sensitive NAATs are recommended for *T. vaginalis* diagnosis
- Specific diagnosis allows for effective treatment
- Coinfection with other STIs is common, implications for testing and patient management